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Supplemental Material to:

Pablo García-Valtanen, Mª del Mar Ortega-Villaizan, Alicia Martinez-Lopez, Regla Medina-Gali, Luis Perez, Simon Mackenzie, Antonio Figueras, Julio M Coll, and Amparo Estepa

Autophagy-inducing peptides from mammalian VSV and fish VHSV rhabdoviral G glycoproteins (G) as models for the development of new therapeutic molecules

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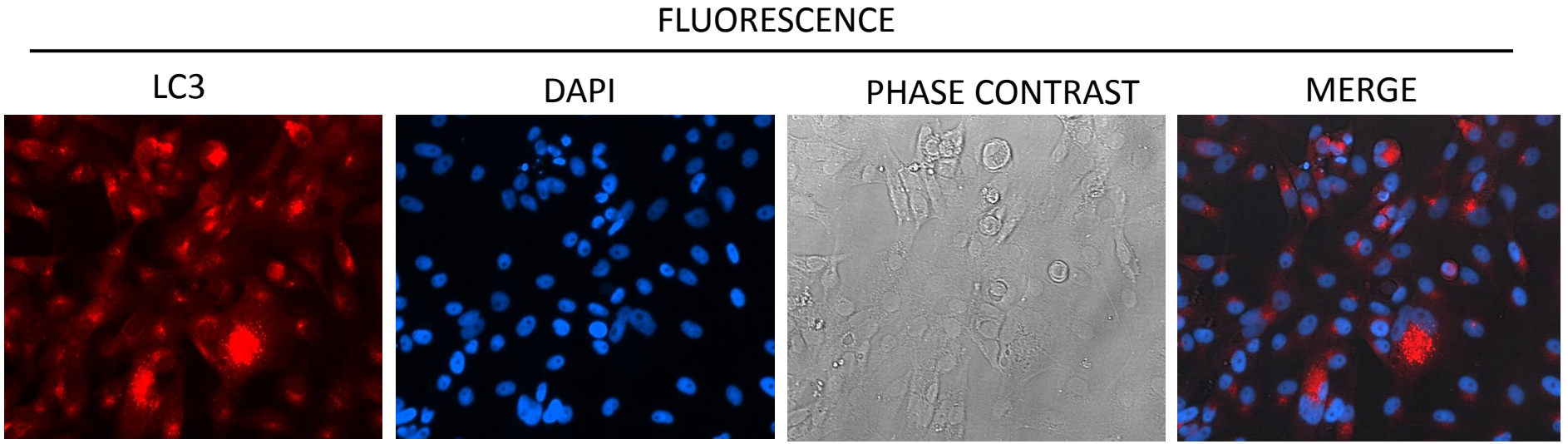


Figure S1

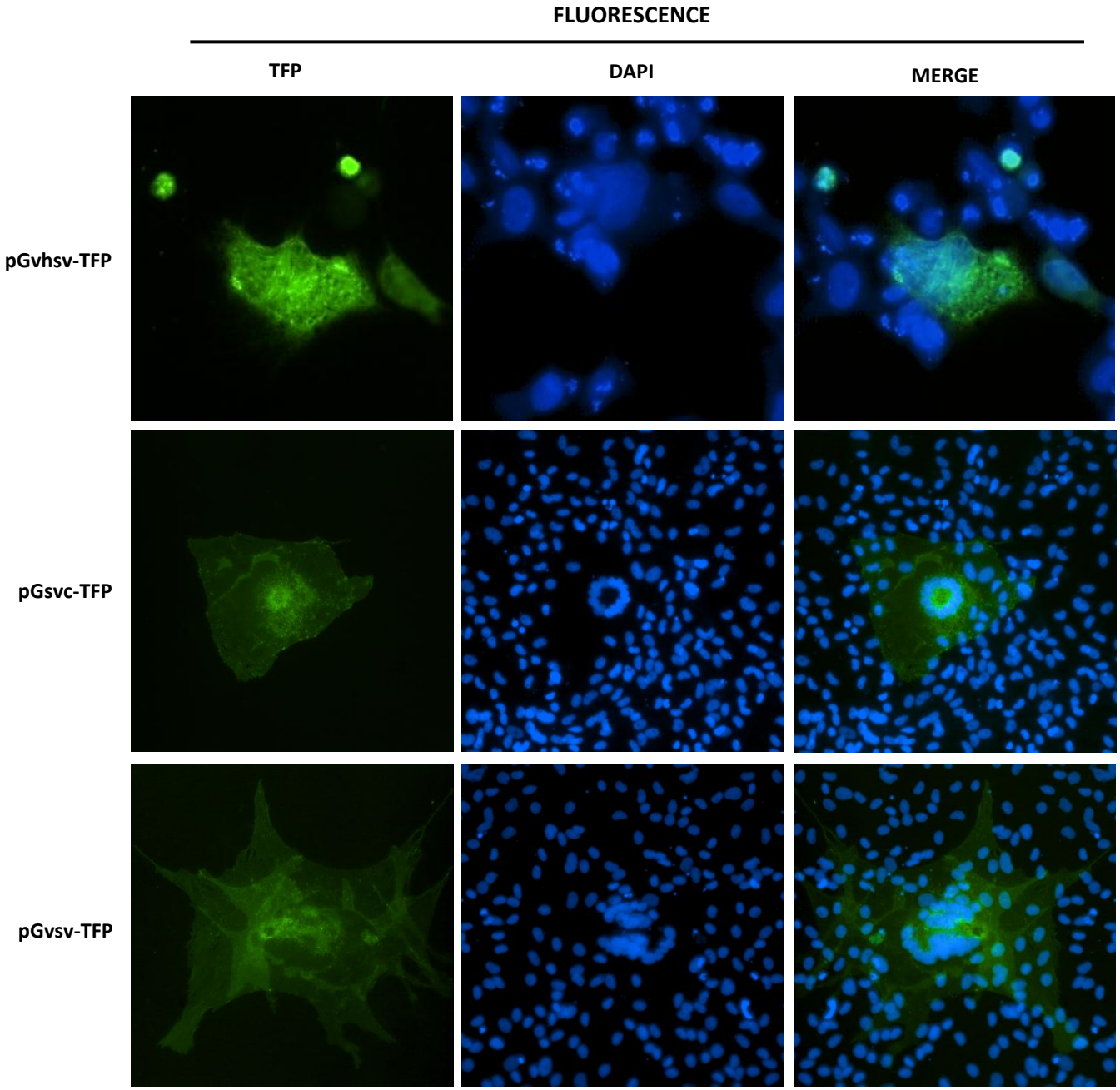


Figure S2

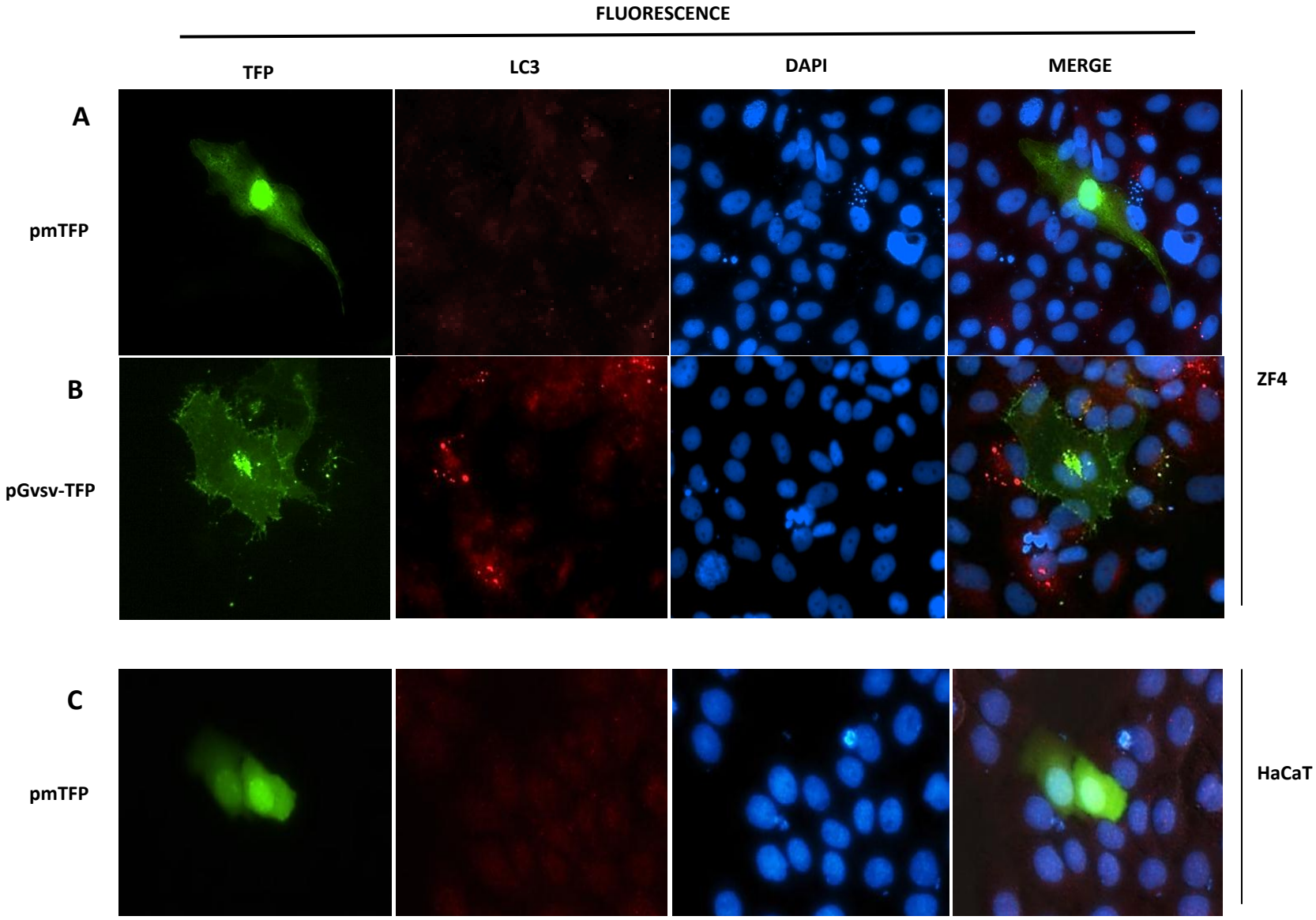

FLUORESCENCE
TFP
LC3
DAPI
MERGE
A
pmTFP
B
pGvsv-TFP
ZF4
C
pmTFP
HaCaT

Figure S3

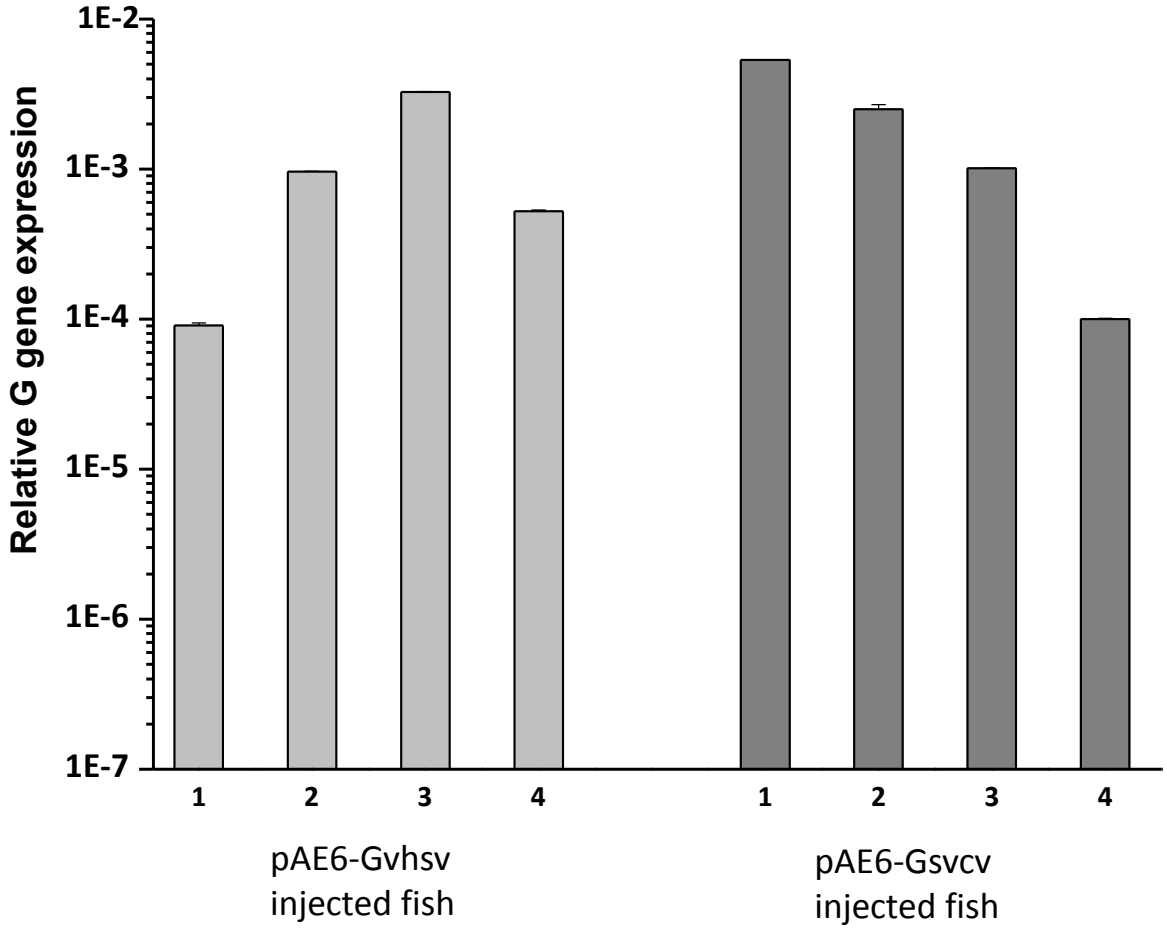
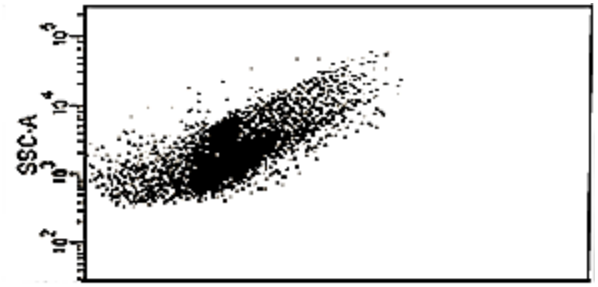

Relative G gene expression
1E-2
1E-3
1E-4
1E-5
1E-6
1E-7
1
2
3
4
1
2
3
4
pAE6-Gvhsv
injected fish
pAE6-Gsvcv
injected fish

Figure S4

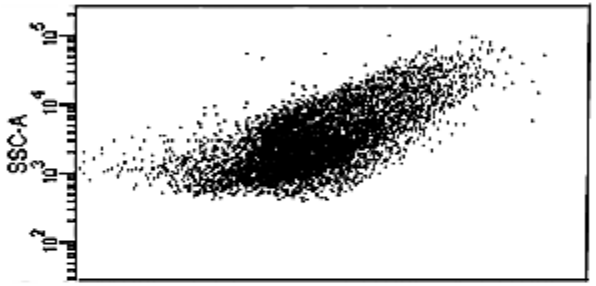
A

HaCat

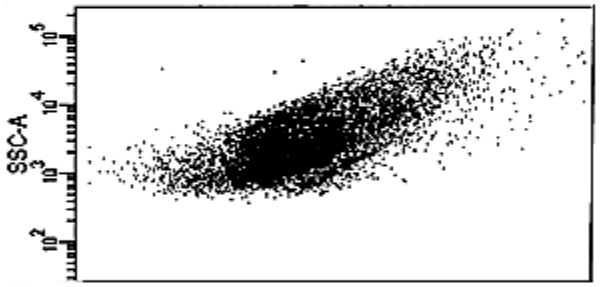
Untreated cells



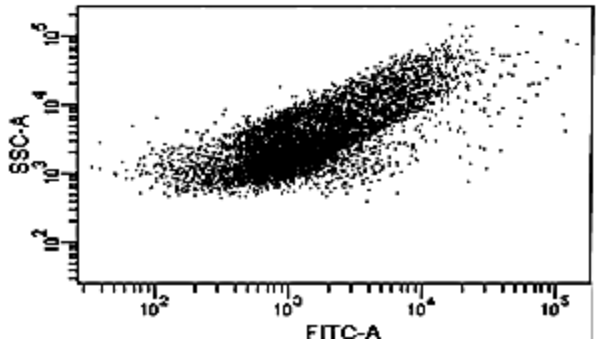
P_{84}-treated cells



P_{344}-treated cells



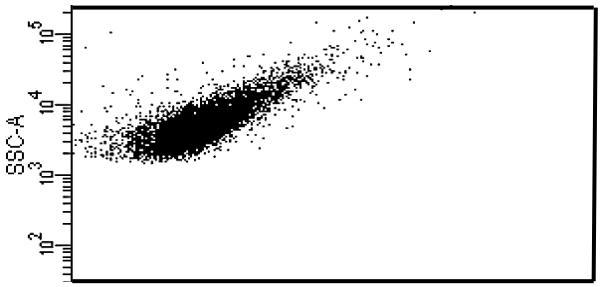
P_{354}-treated cells



B

ZF4

Untreated cells



P_{106}-treated cells

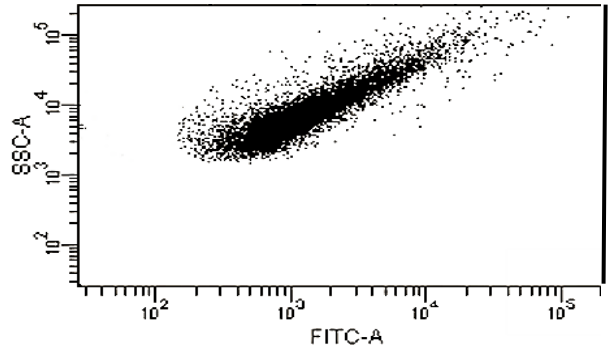


Figure S5

Figure S1. Rapamycin-mediated activation of autophagy in ZF4 cells. ZF4 cells were treated with rapamycin (500 nM) and fixed after 4 h. Then cells were incubated with an antibody anti-LC3 and stained with a fluorophore-conjugated secondary antibody (red fluorescence, LC3). Staining of nuclei was performed by using DAPI (blue fluorescence). Images are representative of the results obtained in 2 independent experiments**.**

Figure S2. pH-dependent membrane fusion in zebrafish cells expressing the rhabdoviral G-TFP1 fusion proteins. ZF4 cells were transfected with 0.5 μg/mL of the constructs pGvhsv-TFP1, pGsvcv-TFP1 or pGvsv-TFP1. Cell nuclei were stained with DAPI. At 72 h post-transfection G-mediated cell membrane fusion was triggered by low pH and syncytia with 5 or more nuclei appeared. Images are representative of the results obtained in 2 independent experiments.

Figure S3. Induction of autophagy by pGvsv-TFP1 and pmTFP1 in ZF4 cells and pmTFP1 in HaCaT cells. The ability to induce autophagy in zebrafish cells was studied by transfecting ZF4 cells with the constructs pGvsv-TFP1 (**A**) and pmTFP1 (**B**). LC3 was stained with an antibody anti-LC3 and fluorescence used to visualize LC3. Also, HaCaT cells were transfected with pmTFP1 and LC3 visualized similarly (**C**). Transfection, DAPI staining and IF assays were carried out as described in Figure 3.

Figure S4. *In vivo* transcript expression of the Gs of SVCV and VHSV after immunization with pAE6-Gsvcv and pAE6-Gvhsv. Adult zebrafish (fish numbered from 1 to 4) were intramuscularly injected with 1.5 μg of pAE6-Gsvcv (dark grey bars) or pAE6-Gvhsv (light grey bars). At 3 d post-transfection, muscle samples, at the site of injection, were excised and the transcript level of the corresponding G measured by RT-qPCR. G transcript levels were calculated with the $2^{-\Delta Ct}$ method, using the *eef1a1l1 gene expression* as endogenous control. Bars represent the mean ± S.D. of 2 measurements for each fish.

Figure S5. Representative dot plots from flow cytometry showing LC3 protein expression in response to VSV and VHSV G pepscan autophagy-inducing peptides. Cells grown in 24-well plates were incubated with 25 μg/mL of the peptides from the Gvsv and Gvhsv pepscans of 15-mer peptides. After 24 h of incubation, LC3 protein expression was measured by flow cytometry using an anti-LC3 antibody. Side scatter (SSC-A) vs LC3 (FITC-A) of (**A**) HaCaT cells untreated or treated with the autophagy-inducing peptides from Gvsv (P84, P344, P354) and (**B**) ZF4 cells untreated or treated with the autophagy-inducing peptide from Gvhsv (P106**).**

Table S1. Primers and probes used in RT-qPCR assays.

Gene	Forward primer (5'-->3´)	Reverse primer (5'-->3')	Probe	Gene bank accession
mxA-B	GGTCTCTGGGAGTCGAAAAGG	AACTCTTTCCCGAGCTTTGGT		NM 182942-AJ544824
eef1a1/1	CCACGTCGACTCCGGAAA	CGATTCCACCGCATTTGTAGA		NM 131263
SVCVgpG	GCTACATCGCATTCCTTTTGC	TCGACCAGATGGAACAAATATGG	ATTGACTCCAACTTAGGAAT*	Z37505.1
VHSVgpG	GGGCCTTCCTTCTACTGGTACTC	CGGAATCCCGTAATTTGGAAT	CTGTTGCTGCAAGGCGTCCCCT**	A10182.1

KEY: ***mxA-B***, mx protein (primers derived from a DNA sequence common to mx isoforms A and B); ***ef1α***, cellular elongation factor 1 alpha; ***SVCVgpG***, SVCV (Fijan strain) glycoprotein G; ***VHSVgpG***, VHSV (isolate VHSV07.71) glycoprotein G; * MGB (quencher)-conjugated probe; ** TAMRA (quencher)-conjugated probe.

Table S1